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PTPA activates protein phosphatase-2A through reducing its phosphorylation at tyrosine-307 with upregulation of protein tyrosine phosphatase 1B

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ABSTRACT

Protein phosphatase-2A (PP2A), an important phosphatase in dephosphorylating tau and preserving synapse, is significantly suppressed in Alzheimer's disease (AD), but the mechanism is not well understood. Here, we studied whether phosphotyrosyl phosphatase activator (PTPA) could activate PP2A by reducing its inhibitory phosphorylation at tyrosine 307 (P-PP2A$_C$). We found that overexpression of PTPA activated PP2A by decreasing the level of P-PP2A$_C$ with reduced phosphorylation of tau, while knockdown of PTPA inhibited PP2A by increasing the level of P-PP2A$_C$ with enhanced tau phosphorylation. We also observed that expression of PTPA could upregulate the protein and mRNA levels of protein tyrosine phosphatase 1B (PTP1B) and simultaneous downregulation of PTP1B eliminated PTPA-induced PP2A activation. Importantly, we also found that the protein level of PTPA is downregulated in the brains of AD patients, and the AD transgenic mouse models with expression of mutant human amyloid precursor protein (hAPP) or the longest human tau (htau), respectively. Our data indicate that PTPA may activate PP2A through activating PTP1B and thus reducing the level of P-PP2A$_C$, therefore upregulation of PTPA may represent a potential strategy in rescuing PP2A and arresting tau pathology in AD.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder. Neurofibrillary tangles (NFTs), which are mainly composed of abnormally hyperphosphorylated tau, are one of pathologic hallmarks in the brain of AD patients [1,2]. While the mechanisms leading to the formation of the tangles are still elusive, an imbalance between protein kinases and phosphatases has been well recognized as the direct cause for the AD-like tau hyperphosphorylation [3]. PP2A accounts for ~70% of total brain tau phosphatase activities and can dephosphorylate tau isolated from AD brains at most of the hyperphosphorylated sites [4]. The activity of PP2A in the AD brains is significantly decreased [5,6]. Compared with other protein phosphatases, the negative correlation between PP2A activity and the levels of abnormal phosphorylated tau in AD brains further supports the dominant role of PP2A in regulation of tau phosphorylation [4–6]. Therefore, it is interesting to dissect the mechanisms that regulate PP2A activity and restoring the activity of PP2A might be a promising target for AD therapy.

PP2A, one of the four major classes (PP1, PP2A, PP2B, and PP2C) of eukaryotic serine/threonine phosphoprotein phosphatases [7,8], is existing as a holoenzymes. The core structure is composed of a 36 kDa catalytic subunit (C subunit) and a constant 65 kDa structural subunit (A subunit or PR65). The PP2A core enzyme associates with a variable regulatory subunit (B subunit) to form the PP2A holoenzyme. It has been reported that PP2A activity is modulated by either non-covalent interactions with regulatory subunits [7], heat stable inhibitors or lipids [9], or covalent post-translational modifications such as methylation and phosphorylation [10–14]. For instance, methylation of PP2A$_C$ at leucine-309, which is catalyzed by methyltransferase (PPMT1), leads to PP2A activation; or unmethylation of PP2A$_C$ at leucine-309, which is catalyzed by methylesterase (PME-1), leads to PP2A inactivation. Phosphorylation of PP2A$_C$ at tyrosine-307 (P-PP2A$_C$), which is regulated by PTP1B and Src, results in PP2A inhibition [15,16]. Moreover, we recently demonstrated that activation of glycogen synthase kinase-3β (GSK-3β) leads to inhibition of PP2A through accumulation of inhibitor-2 of PP2A (I_2^{PP2A}) [17], increase of the level of P-PP2A$_C$ and reduction of PP2A$_C$ protein and mRNA levels [18], decreasing Leu309 methylation by increasing PME-1 protein level and decreasing PPMT1 protein level's and decreasing PP2A$_B$ subunit protein level [19].

Abbreviations: PTPA, protein phosphatase-2A phosphatase activator; GSK-3β, glycogen synthase kinase-3β; PP2A, protein phosphatase-2A; PP2A$_C$, PP2A catalytic subunit; P-PP2A$_C$, phosphorylation of PP2A catalytic subunit at tyrosine-307; M-PP2A$_C$, methylation of PP2A catalytic subunit at leucine-309; DM-PP2A$_C$, unmethylation of PP2A catalytic subunit at leucine-309; AD, Alzheimer's disease; PTP1B, protein tyrosine phosphatase 1B; siRNA, small interference RNA; I_2^{PP2A}, inhibitor-2 of PP2A; pAb, polyclonal antibody; mAb, monoclonal antibody; shRNA, small hairpin RNA; OA, okadaic acid

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The activity of PP2A is also regulated by PP2A phosphatase activator (PTPA), which is initially known as the phosphotyrosyl phosphatase activator. PTPA activates the phosphotyrosyl phosphatase activity of the PP2A core enzyme *in vitro* [20], however, the mechanism by which PTPA activates PP2A is still not well defined. Human PTPA is encoded by a single gene, mapped to chromosome 9q34 [21]. The transcription gives rise to seven different splice variants, four of which are active [22]. Basal expression of the gene is dependent on the ubiquitous transcription factor Yin Yang 1 [23] and functionally antagonized by p53 [24]. PTPA was found to be a highly conserved protein during evolution [25,26] suggesting an important biological function for this protein. However, it is unclear whether PTPA is involved in AD pathogenesis.

Since the phosphorylation of $PP2A_C$ at tyrosine-307 (P-$PP2A_C$) inhibits the PP2A activity [14], we investigated in the present study whether PTPA mediates the P-$PP2A_C$ and the underlying mechanisms. We reported that PTPA mediates the activation of protein phosphatase-2A through reducing the phosphorylation of $PP2A_C$ at tyrosine-307 by protein tyrosine phosphatase 1B.

2. Materials and methods

2.1. Antibodies and regents

Polyclonal antibody (pAb) against PP2A catalytic subunit ($PP2A_C$), pAb against PP2A catalytic subunit phosphorylated at tyrosine-307 site (P-$PP2A_C$) and monoclonal antibody (mAb) against Src were purchased from Millipore (Billerica, MA), mAbs against PP2A catalytic subunit methylated at leucine-309 site (M-$PP2A_C$) and demethylated at leucine-309 (DM-$PP2A_C$) were purchased from Millipore (Billerica, MA), pAbs against PTP1B, and GSK-3β were purchased from Abcam (Cambridge, MA). pAbs against phosphorylated at serine 9 of GSK-3β (pS9-GSK-3β), phosphorylated at tyrosine 216 of GSK-3β (pY216-GSK-3β), pAbs against phosphorylated tau at serine 214 (pS214), against phosphorylated tau at threonine 205 (pT205) or 231 (pT231), against phosphorylated tau at serine 396 (pS396), against phosphorylated tau at serine 404 (pS404), mAbs against total tau (tau-5) and nonphosphorylated tau at threonine 198/199/202 (tau-1)

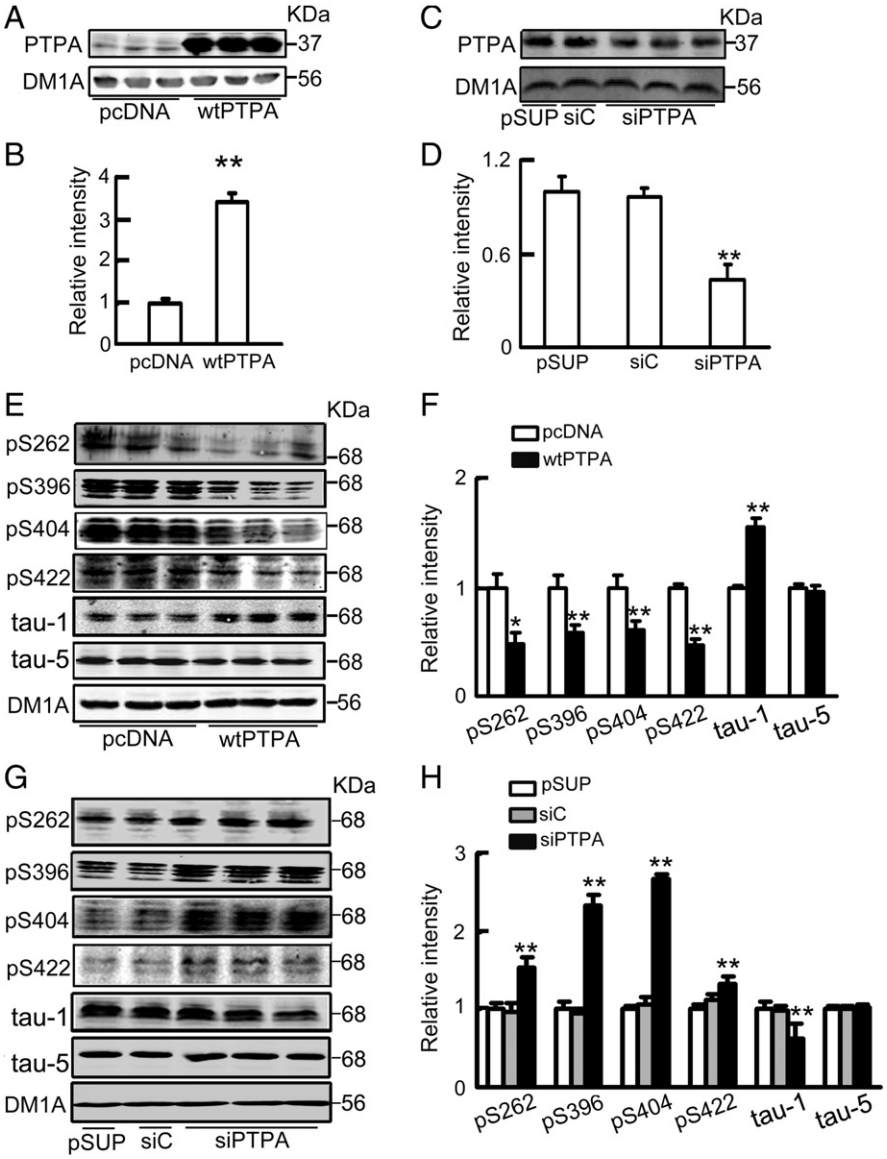

Fig. 1. PTPA regulates tau phosphorylation. The expression of PTPA in HEK293/tau cells was manipulated genetically by transfection of wild type PTPA plasmid (wtPTPA) or pSUP-siPTPA (siPTPA), and pcDNA, pSUP and pSUP-siC (siC) were used as corresponding controls. The levels of PTPA protein or phosphorylated tau were measured by Western blotting (A, C, E, G) and quantitative analysis (B, D, F, H), respectively. The data were presented as means ± S.D. of three independent experiments; *, $p<0.05$, **, $p<0.01$ *vs* control.

were purchased from SAB (Pearland, TX). mAb against total PTPA (PTPA) was purchased from Upstate (Lake Placid, NY), mAb DM1A to α-tubulin was purchased from Sigma (St. Louis, MO). Cell culture media were from Gibico (Grand Island, NY). Lipofectamine 2000 was from Invitrogen (Carlsbad, CA). Okadaic acid was purchased from Sigma (St. Louis, MO). Neurobasal and B27 were from Invitrogen (Carlsbad, CA).

2.2. Construction of plasmids

To knock down PTPA in cells, shRNA oligo sequences were synthesized as follows: 5′-AGCTTCGTTCCCTGTGATCCAGCACTTCAAGAGAGTG CTGGATCACAGGGAACTTTTTTGGAAC-3′, 5′-TCGAGTTCCAAAAAAGTTCC CTGTGATCCAGCACTCTCTTGAAGTGCTGGATCACAGGGAACGA-3′. As control, we used non-sense sequences: 5′-AGCTTCATACCGCTCAGTAGCG ACATTCAAGAGATGTCGCTACTGAGCGGTATTTTTTTGGAAC-3′, 5′-TCGAG TTCCAAAAAAATACCGCTCAGTAGCGACATCTCTTGAA TGTCGCTACTGAGC GGTATGA-3′ [27]. All were purchased as 64-nt ssDNA oligomers composed of both forward and reverse sequences with 9-bp loop structures and 3′ XhoII and 5′ HindIII self-inactivating overhangs. Sense and antisense oligomers (both at 20 μM) were incubated in annealing buffer for 3 min at 90 °C as described [28], then the temperature was lowered in 2 °C/min increments until 5 °C above their respective Tm and then dropped to 4 °C at maximum ramp rates. Annealing shRNA was cloned into pSUPER (pSUP), a mammalian expression vector that directs the synthesis of siRNAs: pSUPER-siPTPA (pSUP-siPTPA) and pSUPER-siCon (pSUP-siC).

pSilencer plasmid was purchased from GeneChem Co., Ltd (Shanghai, China), and sequences of PTP1B (5′-UAGGUACAGAGACGUCAGU-3′) were selected to generate pSilencer constructs that produce small hairpin RNA (shRNA), which was then spontaneously processed *in vivo* into siRNA.

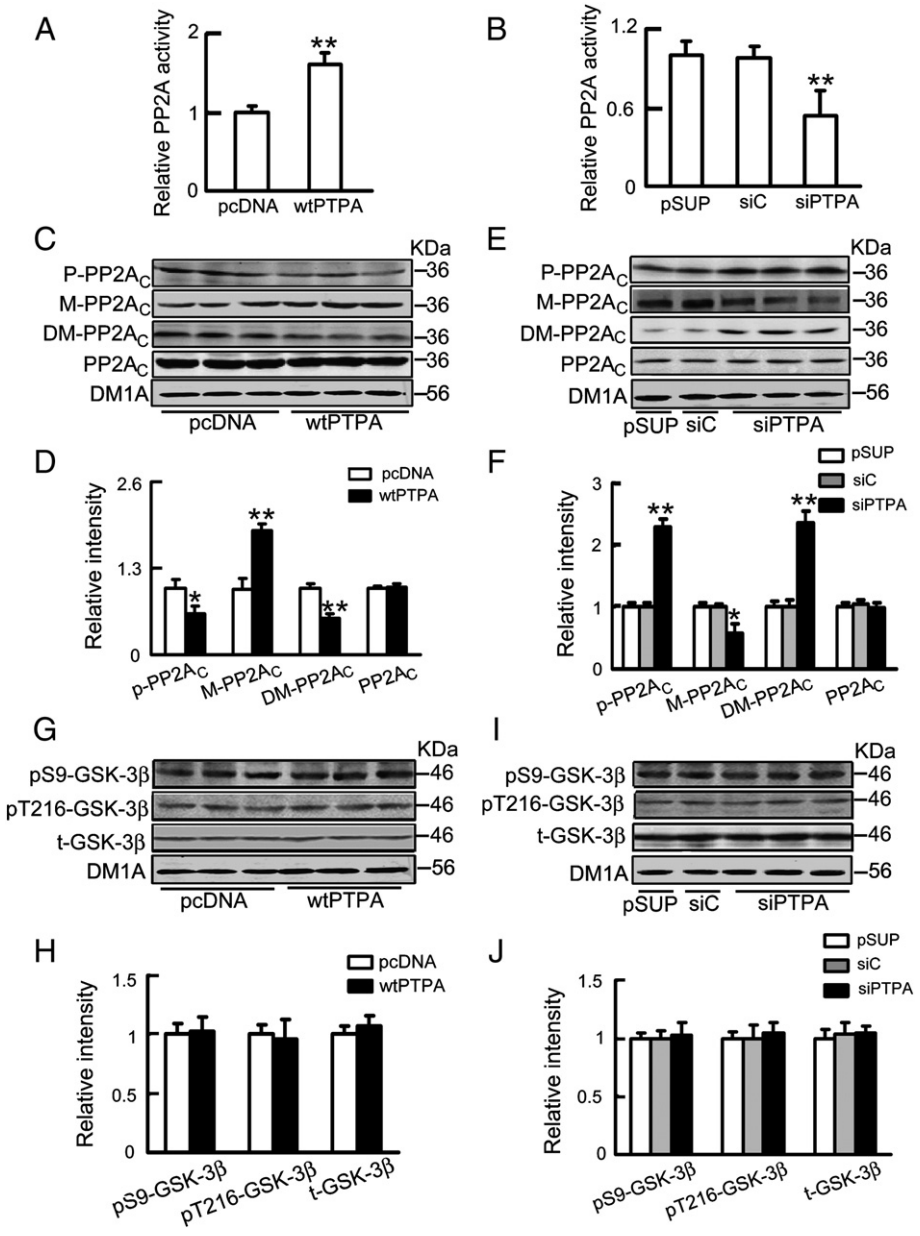

Fig. 2. PTPA regulates PP2A activity with no effect on GSK-3β. The biochemical activity and the activity-dependent modifications of PP2A or GSK-3β were measured when PTPA was overexpressed or knocked down in HEK293/tau cells. PP2A activity was measured by protein phosphatase activity assay (A and B). The levels of total $PP2A_C$, the methylated ($M\text{-}PP2A_C$), demethylated ($DM\text{-}PP2A_C$), phosphorylated levels of $PP2A_C$ ($P\text{-}PP2A_C$) (C–F), the total GSK-3β (t-GSK-3β) and the phosphorylated GSK-3β (pS9-GSK-3β, pT216-GSK-3β) (G–J) were measured by Western blotting (C, E, G, I) and quantitative analysis (D, F, H, J), respectively. The data were presented as means ± S.D. of three independent experiments; *, $p<0.05$, **, $p<0.01$ *vs* control.

The human PTPA cDNA sequence was obtained by RT-PCR amplification and cloned into the eukaryotic expression vector pcDNA 3.1(+). All plasmids were confirmed by enzyme digestion and DNA sequencing.

2.3. Cell culture and transfection

The human embryonic kidney 293 (HEK293/tau) cells stably transfected with the longest isoform of recombinant human tau (tau441) were used for the study. HEK293/tau cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS, GIBCO, MI) in a humidified atmosphere of 5% CO_2 in air at 37 °C. The cells were plated into six-well plates overnight and plasmids were transfected the next day using Lipofectamine 2000 according to the manufacturer's instruction (Invitrogen, CA).

The primary neurons from E18 rat hippocampus were seeded at 30,000–40,000 cells per well on 6-well chamber slides coated with Poly-D-Lysine/Laminin (BD) in neurobasal medium supplemented with 2% B27/0.5 mM glutamine/25 mM glutamate. Half the culture medium was changed every 3 days with neurobasal medium supplemented with 2% B27 and 0.5 mM glutamine. All cultures were kept at 37 °C in a humidified 5% CO_2 containing atmosphere. More than 90% of the cells were neurons after they were cultured for 7–17 days *in vitro* (div); this was verified by positive staining for the neuronal specific markers microtubule-associated protein-2 (MAP2, dendritic marker). At 7 div, the neuron transfections were performed according to the manufacturer's instruction (Invitrogen), and the ratio of the plasmids to Lipofectamin 2000 was 1:2.

2.4. Western blotting and immunohistochemistry

For Western blotting, the cell or brain extracts were mixed with sample buffer containing 50 mM Tris–HCl (pH 7.6), 2% SDS, 10% glycerol, 10 mM dithiothreitol and 0.2% bromophenol blue and boiled for 5 min. Human tau transgenic mice (htau) and its littermate control (endogenous tau knockout, tau$^{-/-}$) were purchased from Jackson Lab. The proteins were separated by 10% SDS-PAGE, transferred onto the nitrocellulose membrane and incubated with primary antibody at 4 °C overnight. Immune complexes were detected with appropriate secondary antibodies and enhanced chemiluminescence (ECL) and quantitatively analyzed by Kodak Digital Science 1D software (Eastman Kodak Company, New Haven, CT). The relative intensity was expressed as sum optical density.

For immunohistochemical studies, brain sections of AD patients were gifts from Prof. Iqbal K (NYS Institute for Basic Research, Staten Island, NY, USA). Sections were incubated at 4 °C overnight with primary antibodies. Immunoreaction was developed using HistostainTM-SP kits and visualized with diaminobenizidine. The sections were observed using a microscope (Olympus BX60, Tokyo). For immunofluorescence, cells were cultured on chamber slides. After various treatments, the cells were fixed for 40 min with 4% paraformaldehyde in PBS (pH 7.4) and permeabilized for 10 min at room temperature in PBS containing 0.5% Triton X-100. Cells were blocked with 5% bovine serum albumin (BSA) for 40 min, and further incubated with primary antibody at 4 °C overnight, and then incubated for 1 h at 37 °C with Rhodamine Red-X- or Oregon Green 488-conjugated secondary antibodies (1:1000; Molecular Probes). All fluorescence images were captured with a Zeiss LSM 510 inverted fluorescence microscope or a Zeiss LSM 710 laser-scanning confocal fluorescence microscope.

2.5. RT-PCR

Total RNA was isolated using Trizol™ according to the manufacturer's instruction (Invitrogen, CA). Then total RNA (3 μg in 25 μl) was reversely transcribed and the produced cDNA (1 μl) was used to detect the transcripts. For PTP1B, the following primers were used: 5′-GAGATTAC TTTGTCCCGCTTAT-3′ (forward primer) and 5′-ATCCCTTCGTCCCTGTGG -3′ (reverse primer). For GAPDH, 5′GAAGGTGAAGGTCGGAGTC3 ′ (forward primer) and 5′GAAGATGGTGATGGGATTTC3 ′ (reverse primer) were used. The PCR products were separated on 1.5% agarose gels and stained with GoldView. The cDNA bands were visualized under UV transillumination and quantitatively analyzed using software BioCaptMW V.10 (Vilber Lourmat, Marne-La-Vallee Cedex 1).

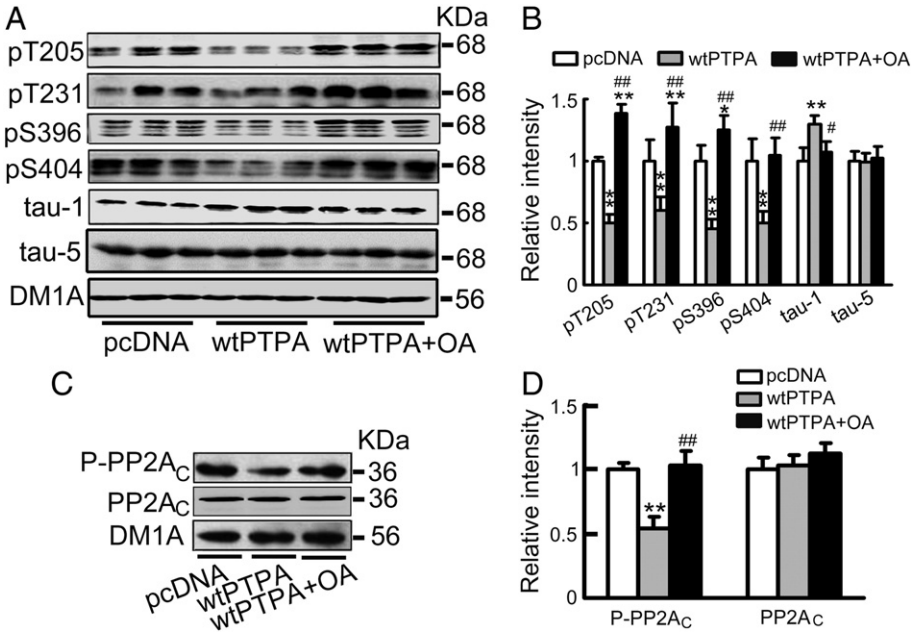

Fig. 3. OA reverses the PTPA-suppressed tau phosphorylation. HEK293/tau cells were transfected with wild type PTPA plasmid (wtPTPA) or the vector (pcDNA) for 24 h, then treated with OA (100 nM) for 4 h, levels of the hyperphosphorylated tau at multiple epitopes (A, B) and the phosphorylated levels of PP2A$_C$ (P-PP2A$_C$) (C, D) were detected by Western blotting and quantitative analysis, respectively. The data were presented as means ± S.D. of three independent experiments; **, $p<0.01$ *vs* pcDNA; #, $p<0.05$, ##, $p<0.01$ *vs* wtPTPA.

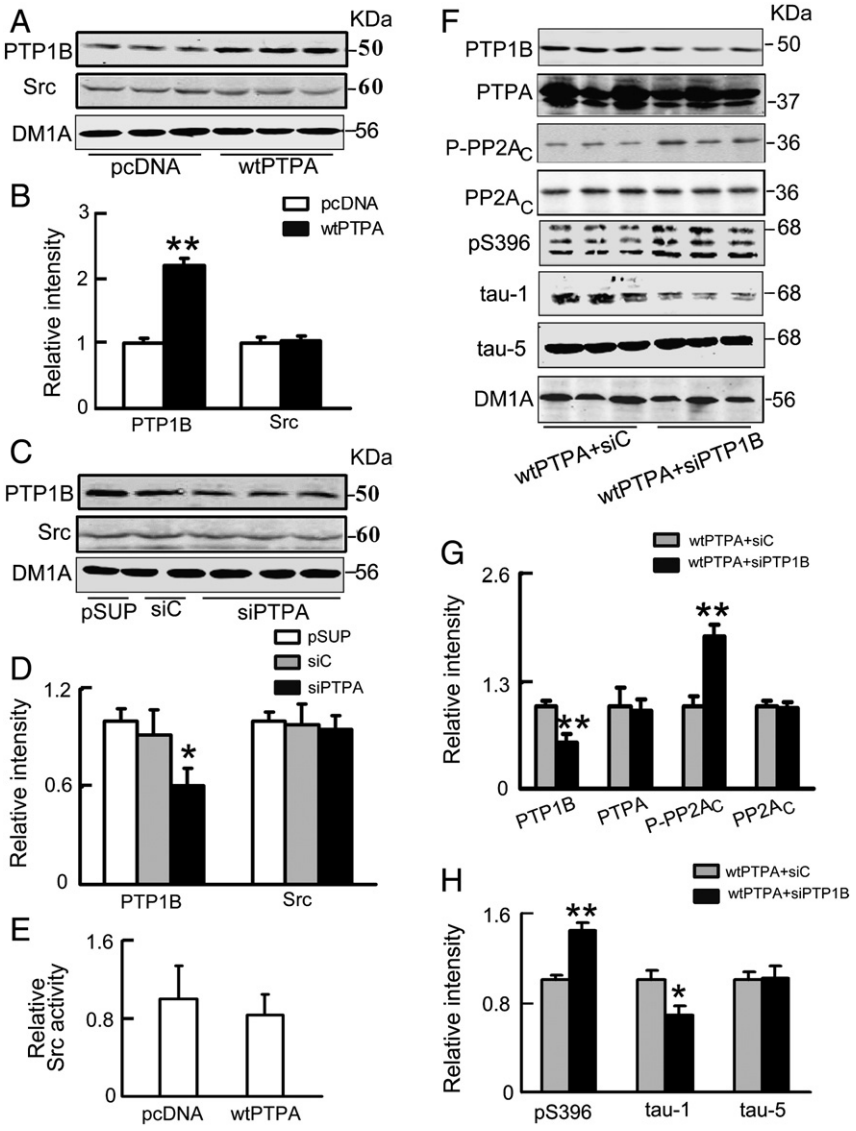

Fig. 4. Knockdown of PTP1B abolishes PTPA-induced dephosphorylation of $PP2A_C$ at Tyr307. (A–E) HEK293/tau cells were transfected with wtPTPA or siPTP1B or the control vectors in HEK293/tau cells for 48 h, and then the level and activity of PTP1B or Src were measured by Western blotting (A, C), quantitative analysis (B, D), and chemical assay (E), DM1A as a loading control. (F–G) HEK293/tau cells were co-transfected with wtPTPA and siPTP1B or siC for 48 h, then the levels of PTP1B, PTPA, $PP2A_C$ and P-$PP2A_C$ are measured by Western blotting (F) and quantitative analysis (G-H), respectively. The data were presented as means ± S.D. of three independent experiments; *, $p<0.01$, **, $p<0.01$ *vs* control or wtPTPA + siC.

2.6. Protein phosphatase and Src activity assay

To detect PP2A activity, the cell lysates were prepared by adding phosphatase storage buffer (2 mM EGTA, 5 mM EDTA, 0.5 mM PMSF, 50 mM Tris–Cl (pH 7.4), 1:200 protease inhibitor cocktail) and endogenous phosphates was removed by using the Spin Column. The activity of PP2A in the extract was assayed using a serine/threonine phosphatse assay system (Promega, MA) according to the manufacturer's protocol.

The Src activity was measured by using a Src kinase assay kit (GENMED SCIENTIFICS INC., Shanghai, China) according to the manufacturer's instruction.

2.7. Statistical analysis

Data were analyzed with SPSS 13.0 statistical software. The one-way analysis of variance procedure followed by least significant difference *post hoc* tests was used to determine the statistical significance of differences of the means.

3. Results

3.1. PTPA negatively regulates tau phosphorylation

To explore the effects of PTPA on tau phosphorylation, we transfected pcDNA-PTPA or pSUP-siPTPA plasmid into HEK293/tau cells. We first measured the protein level of PTPA at 48 h after the transfection by Western blotting. The levels of PTPA increased to ~368% when PTPA was overexpressed (Fig. 1A and B), while the level of PTPA decreased to ~41.5% of the control level after PTPA knockdown (Fig. 1C and D). Overexpression of PTPA reduced significantly the phosphorylation levels of tau at Ser262 (pS262), Ser396 (pS396), Ser404 (pS404), Ser422 (pS422) and Ser198/199/202 (tau-1) sites (tau-1 antibody is specific for tau dephosphorylated at this specific site and its increase suggests the increased dephosphorylation at this site) (Fig. 1E and F). In contrast, downregulation of PTPA dramatically enhanced tau phosphorylation at the same sites (Fig. 1G and H). There was no significant change of total tau (tau-5). These data indicate that PTPA regulates tau phosphorylation.

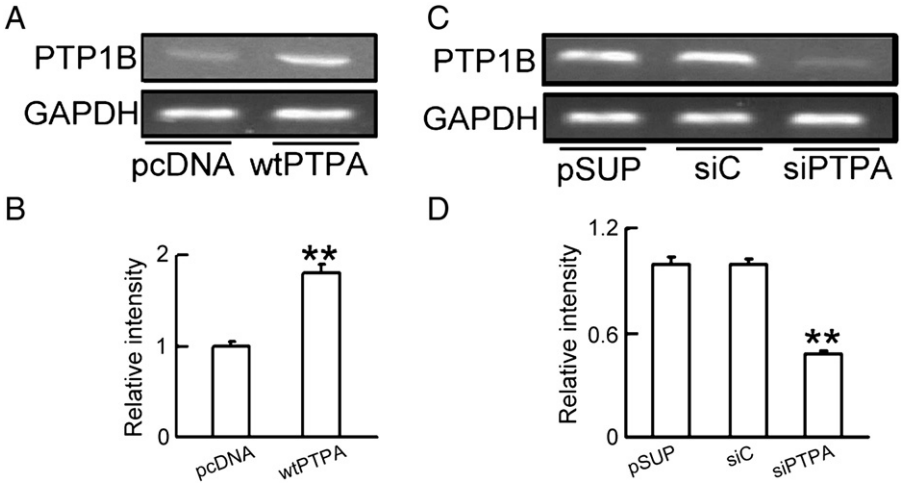

Fig. 5. PTPA increases PTP1B transcription. The expression of PTPA in HEK293/tau cells was manipulated genetically by transfection of wtPTPA or pSUP-siPTPA (siPTPA), and pcDNA, pSUP and pSUP-siC (siC) were used as controls. PTP1B mRNA level was detected by RT-PCR with GAPDH as a loading control. The data were presented as means ± S.D. of three independent experiments; **, $p<0.01$ *vs* control.

3.2. Activation of PP2A is responsible for PTPA-mediated tau phosphorylation

PP2A is a crucial phosphatase in dephosphorylating tau, we therefore first measured PP2A activity. We found that overexpression of PTPA activated PP2A, whereas knockdown of PTPA inhibited PP2A activity (Fig. 2A and B). Then, we investigated how PTPA regulates PP2A activity. To this end, we detected the levels of methylation or phosphorylation of PP2A catalytic subunit ($PP2A_C$) because both of these modifications modulate PP2A activity. We observed that the inhibitory phosphorylation of $PP2A_C$ at tyrosine-307 (P-$PP2A_C$) decreased significantly in the cells overexpressing PTPA (Fig. 2C and D), while the level of P-$PP2A_C$ increased in PTPA-depleted cells (Fig. 2E and F). We also discovered that overexpression of PTPA increased the levels of methylated $PP2A_C$ at leucine-309 (M-$PP2A_C$, active form) with reduction of the demethylated $PP2A_C$ (DM-$PP2A_C$, inactive form) (Fig. 2C and D). In contrast to PTPA overexpression, knockdown PTPA had inverse effects on $PP2A_C$ methylation (Fig. 2E and F). The total level of $PP2A_C$ was not changed. These results indicate that PTPA activates PP2A by regulatng the methylation and/or phosphorylation of $PP2A_C$.

Glycogen synthase kinase-3β (GSK-3β) is a key tau kinase [4,5], GSK-3β also inhibits PP2A [17–19]. Thus, we studied whether PTPA influences GSK-3β activity. We found that neither overexpression nor knockdown of PTPA changed the levels of phospho-GSK-3β at

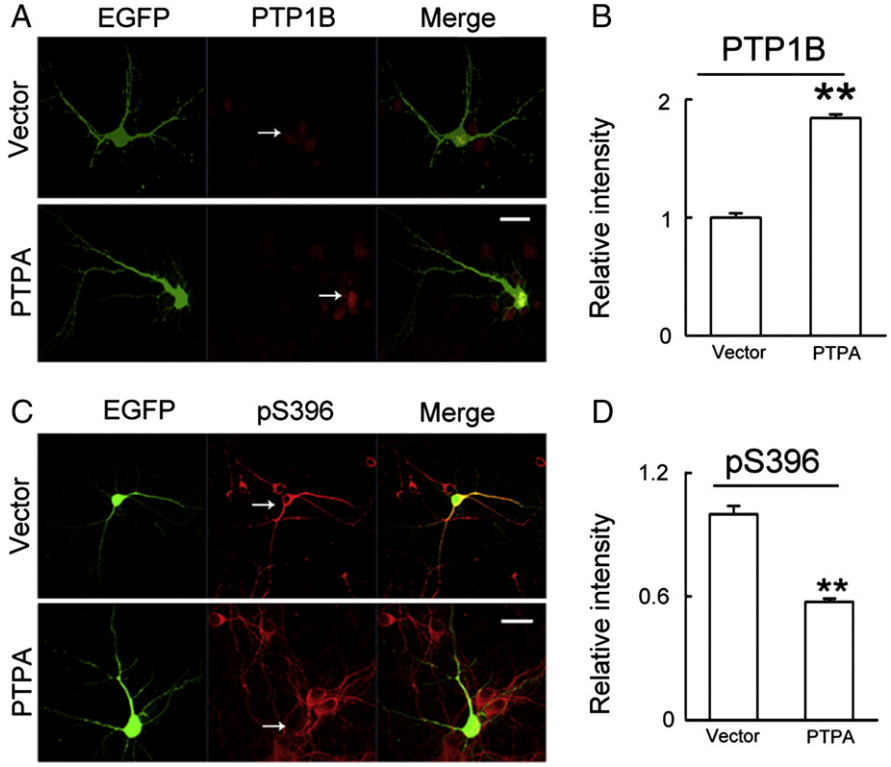

Fig. 6. PTPA increases PTP1B and decreased pS396 in primary hippocampal neuron. The eGFP-labeled PTPA were transiently transfected into rat primary hippocampal neurons for 48 h, and then PTP1B (A) and tau phosphorylated at Ser396 site (pS396) (C) were detected by immunofluorescence staining and quantitative analysis (B, D). Arrows indicate transfected neurons, and the data were presented as means ± S.D of ~15 neurons with EGFP out of ~180 neurons, respectively; **, $p<0.01$ *vs* control. Scale bar = 20 μm.

serine-9 (pS9, inactive form) or tyrosine-216 (pY216, active form) (Fig. 2G-J), indicating that PTPA does not affect GSK-3β.

To further verify whether PTPA decreases tau phosphorylation by activation of PP2A, we treated PTPA expressing cells with PP2A inhibitor okadaic acid (OA) for 24 h. We observed that OA treatment attenuated the PTPA-induced tau dephosphorylation at several sites with no obvious change in total tau level probed by R134d antibody (Fig. 3A and B). We also found that, although PTPA overexpression decreased P-PP2A$_C$, P-PP2A$_C$ increased while combined with OA treatment (Fig. 3C and D).

3.3. PTPA modulates the phosphorylation of PP2A$_C$ by PTP1B

Since phosphorylation of tyrosine-307 is regulated by PTP1B (a tyrosine phosphatase) and Src (a tyrosine kinase) [15,16], we detected the levels of PTP1B and Src in cells with overexpression or knockdown of PTPA. We found that overexpression of PTPA increased the level of PTP1B (Fig. 4A–D), whereas knockdown of PTPA decreased PTP1B level with no obvious changes in Src level (Fig. 4A–D) or activity (Fig. 4E).

To further confirm the involvement of PTP1B in dephosphorylation of PP2A$_C$ at tyrosine-307, we used siRNA to knock down the level of PTP1B. We observed that downregulation of PTP1B abolished the PTPA-induced dephosphorylation of PP2A$_C$ (Fig. 4F and G). This result suggests that PTPA downregulates phosphorylation of PP2A$_C$ at tyrosine-307 through upregulating PTP1B but not Src.

To explore the mechanisms underlying PTPA-induced upregulation of PTP1B protein, we performed semi-quantitative PCR to measure the mRNA levels of PTP1B. We found that overexpression of PTPA increased the mRNA level of PTP1B, whereas knockdown of PTPA significantly decreased the level of PTP1B mRNA (Fig. 5A-D). These data indicate that PTPA may increase PTP1B protein through upregulating mRNA transcription.

To confirm the results, we did transiently transfected pcDNA-PTPA into rat primary hippocampal neurons cultured for 7 days *in vitro* (*div*). We also observed that neurons expressing PTPA increased the immunostaining of PTP1B, and decreased immunostaining of pS396 compared with neurons expressing GFP control (Fig. 6).

3.4. PTPA protein is decreased in transgenic mouse models and AD brain

To investigate whether PTPA is involved in AD pathogenesis, especially in tau phosphorylation, we detected the levels of PTPA protein in the brains of 11 month-old tg2576 mice harboring the human amyloid precursor protein 695 with Swedish double mutation (hAPP) (HuAPP695; K670N/M671L, Jackson Lab), 11 month-old human tau transgenic mice (hTau) (STOCK *Mapt*$^{tm1(EGFP)Klt}$ Tg(MAPT) 8cPdav/J, Jackson Lab), and AD patients. We found that PTPA protein was decreased dramatically in the brains of the transgenic mice and AD patients, when compared with the age-matched controls (Fig. 7).

4. Discussion

PP2A is the most important phosphatase implication in dephosphorylating the abnormally hyperphosphorylated tau proteins isolated from AD brains [29,30]. The activity of PP2A is significantly suppressed in brains of AD patients [5,6], but the upstream factors leading to the inhibition of PP2A is not fully understood. Recently, we have reported that activation of GSK-3β, a crucial tau kinase that is activated in the AD brain, inhibits PP2A through upregulating I_2^{PP2A} [17], increasing the inhibitory tyrosine-307 phosphorylation and decreasing the expression of PP2A [18]. In the present study, we determine whether PTPA regulates PP2A activity by detecting the phosphorylation of PP2A at tyrosine-307, which inhibits PP2A activity. We observed

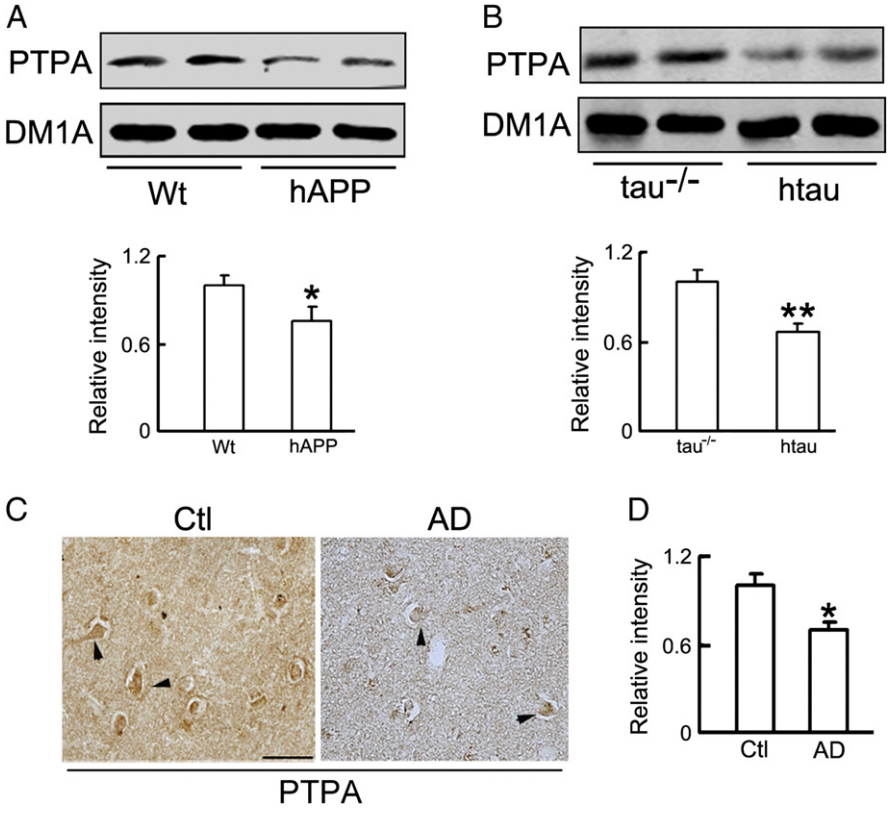

Fig. 7. PTPA protein level decreases in brains of transgenic mice or AD patient. (A–B) The level of PTPA protein in 11 month-old non-transgenic (Wt), hAPP, htau or tau$^{-/-}$ transgenic mice was measured by Western blotting and quantitative analysis (n = 4). (C–D) PTPA levels in the brain of AD patients or the controls were detected by immunohistochemistry (arrowheads indicate immunostaining of PTPA in the neurons) and quantitative analysis (n = 3). The data were presented as means ± S.D. of three independent experiments; *, $p<0.05$ *vs* Wt, **, $p<0.01$ *vs* tau$^{-/-}$ mice. Scale bar = 50 μm.

that upregulation of PTPA reduced this phosphorylation, whereas downregulation of PTPA enhanced it. Together, these data indicate that PTPA directly regulated PP2A activity through regulating the activity-dependent phosphorylation of tyrosine-307.

PTPA, a ubiquitous [31] and highly conserved protein [26], is first named as phosphotyrosyl phosphatase activator. Previous *in vitro* study has reported that PTPA has peptidyl prolyl cis/trans-isomerase (PPIase) activity and regulates the isomerization of Pro-190 on human $PP2A_C$, therefore activating PP2A [32]. Additional study has demonstrated that the interaction of PTPA with hetero-dimeric PP2A A-C results in activation and alteration of substrate specificity of PP2A [33]. Moreover, recent studies have reported that PTPA can physiologically function to reactivate the inactive form of PP2A (PP2Ai) [34–36], which binds to PME-1 to form a stable complex. This finding led to the renaming of PTPA as the PP2A phosphatase activator.

In the present study, we showed that genetic upregulation of PTPA level increased PP2A methylated at leucine-309. We also found that over expression of PTPA can reduce tau phosphorylation, indicating that PTPA positively regulates PP2A Ser/Thr activity. Moreover, we firstly discovered that PTPA activates PP2A activity by decreasing the level of PP2A phosphorylated at tyrosine-307.

To explore the molecule(s) that may mediate the effects of PTPA on PP2A phosphorylation at tyrosine-307, we detected the level of PTP1B, the first purified protein tyrosine phosphatase that can dephosphorylate PP2A at tyrosine-307 [37]. We found that upregulation of PTPA increased the protein and mRNA levels of PTP1B. Furthermore, knockdown of PTP1B by siRNA almost abolished the PTPA-induced PP2A dephosphorylation. These data strongly suggest that PTPA may regulate the tyrosine phosphorylation of PP2A through PTP1B. A previous study also demonstrated that overexpression of PTP1B enhanced PP2A activity *via* decreasing the level of phosphorylated $PP2A_C$ at tyrosine-307 [38]. Previous studies have shown that Src, a tyrosine kinase, can phosphorylate $PP2A_C$ at tyrosine-307, and thus inhibit PP2A activity [38,39]. Therefore, we also investigated whether Src is involved in the PTPA-induced PP2A dephoshorylation at tyrosine-307. We found that either the protein level or the activity of Src was not changed by PTPA.

PTPA induced cell apoptosis in HCT116 human colorectal carcinoma and the OK opossum kidney cell lines [40]. Incubation of cells with the PP2A inhibitor okadaic acid does not prevent PTPA-induced apoptosis, indicating that PTPA is unlikely to mediate its proapoptotic effect *via* PP2A [40]. PTP1B is a negative regulator of tyrosine kinase growth factor signaling, overexpression of PTP1B induced brown adipocyte, chondrocytes and hepatocyte apoptosis [41–43]. In the present study, we were surprised to find that overexpression of PTPA has no effect on the viability of HEK293/tau cells (Fig. S1), the mechanism needs further investigation.

Finally, to understand the pathological significance of PTPA in AD pathogenesis, we found that the levels of PTPA are decreased in the brains of hAPP, htau transgenic mice and AD patient. Taken together, we have demonstrated in the present study that PTPA enhanced PP2A activity through reducing the phosphorylation of $PP2A_C$ at tyrosine-307 by PTP1B. Moreover, downregulation of PTPA might be partially responsible for PP2A suppression, and therefore contributes to tau phosphrylation in AD pathogenesis. Restoration of PTPA in AD brains may result in activation of PP2A, which may represent a powerful strategy in preventing/reversing tau hyperphosphorylation and accumulation.

Conflicts of interest statement

The authors declare that they have no potential conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2013.02.005.

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